



ISOLATION AND CHARACTERIZATION OF *ACINETOBACTER* SP. STRAIN THAT GROWS FAST ON ACETATE AS CARBON SOURCE.

Juan Carlos Sigala^a, Brisa Paola Suárez^b, Álvaro Lara^a, Sylvie Le Borgne^a

^a Universidad Autónoma Metropolitana, Unidad Cuajimalpa, Departamento de Procesos y Tecnología, México D.F. ^b Instituto Tecnológico de Acapulco, Guerrero México.

jsigala@correo.cua.uam.mx

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Introduction. The bacterium under study was isolated as a contaminant from the laboratory ambience on minimal medium plates with acetate.

The goal of this work was to identify the isolate by analyzing a conserved fragment of its 16S rDNA and to determine the kinetic parameters of cultivations in bioreactor with minimal medium and acetate 6 g/l as the sole carbon source.

Methods. Single colonies on LB plates were used to inoculate mineral medium with 3 g/l acetate. Genomic DNA was obtained and a fragment of 16S rDNA was amplified by PCR, cloned in a suitable vector and sequenced. Alignment was used to determine the identity of the strain [1]. Shake flask cultivations were conducted in minimal medium with acetate 3 and 6 g/l as carbon source, 250 rpm agitation and 30°C incubation. Bioreactor cultures were performed with a working volume of 0.6 L, 1 vvm, pH 7, 30 °C, dissolved oxygen tension above 20% [2], and the same medium with 6 g/l acetate. Biomass formation was followed spectrophotometrically and gravimetrically. Acetate concentration was measured by HPLC.

Results. The isolated microorganism was identified as *Acinetobacter* sp. by comparison of a conserved fragment of its 16S rRNA nucleotide sequence. This strain, designated as A122, had an unusual high specific growth rate ($\mu = 0.9 \text{ h}^{-1}$) on shake flask cultivations with minimal medium and 3 g/l acetate as carbon source. A reference strain, *Acinetobacter baylyi* ADP1 [3], had a $\mu = 0.7 \text{ h}^{-1}$ in the same conditions. Only for strain A122, when acetate concentration was increased to 6 g/l, growth decreased in the shake flask by the end of the exponential phase possibly due to oxygen limitation caused by the high growth rate and poor mass transfer (fig.1). To guarantee oxygen supply and to control pH, A122 strain was cultivated in a bioreactor and could grow exponentially at high constant rate with 6 g/l (Table 1) until the substrate was exhausted. Additional kinetics parameters were determined from the bioreactor cultures of both strains (Table 1).

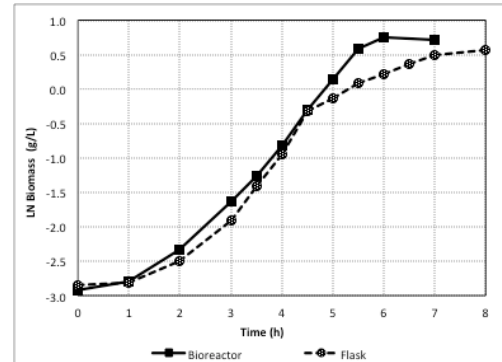


Fig.1 Growth of strain A122 on bioreactor and shake flask with minimal medium and 6 g/l acetate.

Table 1. Kinetic parameters of strains *A. baylyi* ADP1 and A122 from bioreactor cultivations with 6 g/l acetate.

Parameter	<i>A. baylyi</i> ADP1	A122
μ (h ⁻¹)	0.44	0.89
$Y_{x/s}$ (g DCW/gAce)	0.18	0.43
q_s (gAce /gDCW h)	2.39	2.06

Conclusions.

Our isolate (A122) was identified as *Acinetobacter* sp. This isolate grows fast on minimal medium with acetate as the sole carbon source ($\mu = 0.9 \text{ h}^{-1}$). Cultivation conditions in bioreactor were established to sustain fast growth of this strain on minimal medium with 6 g/L of acetate. Kinetic parameters from cultivations on bioreactor show that A122 strain catabolizes acetate more efficiently as compared with the well-known *Acinetobacter baylyi* ADP1 strain. We are exploring currently if the isolated strain has the aromatic degradative capabilities seen in *A. baylyi* [4], which could be useful for bioremediation studies in the future.

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